Available online at www.sciencedirect.com

SCIENCE DIRECT

Virology 344 (2006) 77 – 87

VIROLOGY

www.elsevier.com/locate/yviro

Conserved molecular systems of the Baculoviridae

Kazuhiro Okano, Adam L. Vanarsdall, Victor S. Mikhailov, George F. Rohrmann *

Department of Microbiology, Nash Hall Room 220, Oregon State University, Corvallis, OR 97331-3804, USA

Received 1 September 2005; accepted 10 September 2005

Abstract

Although the Baculoviridae are a large and diverse family of viruses, they are united by a number of shared features that form the basis for their unique life cycle. These include the mechanism of cell entry, genome replication and processing, and late and very late gene transcription. In this review, the molecular systems that are conserved within the Baculoviridae and that are responsible these processes are described.

Contents

* Corresponding author. Fax: +1 541 737 0496.
E-mail address: rohrmanng@orst.edu (G.F. Rohrmann).

0042-6822/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.virol.2005.09.019

The Baculoviridae

Baculoviruses are occluded viruses with genomes consisting of double-stranded, covalently closed circular (ccc) DNA molecules, and are pathogenic for insects predominantly of the orders Lepidoptera, Hymenoptera, and Diptera. The Baculoviridae have been divided into two genera based on occlusion body morphology. The nucleopolyhedroviruses (NPVs) produce large occlusion bodies containing numerous rod-shaped enveloped virions in the nuclei of infected cells (Rohrmann, 1999). The other genera, the granuloviruses (GVs) (Winstanley and O'Reilly, 1999), produce small granular occlusion bodies that normally contain a single virion and are only found in the Lepidoptera. During GV infection, the cytoplasm and nucleus of infected cells appear to merge and the occlusion bodies are located throughout the cell. The recent knowledge gained from the completion of baculovirus genome sequences from a number of GVs and NPVs pathogenic for Lepidoptera, Hymenoptera, and Diptera has provided major insights into their diversity. The purpose of this review is to highlight themes of baculovirus molecular biology that are likely shared between all members of the family. Since most research has been done on the *Autographa californica* multinucleocapsid (AcMNPV) because of its ease of manipulation and growth in cell culture, we are assuming that the presence of common sets of genes reflects related methods for carrying out similar processes in all baculoviruses.

Baculovirus evolution

Evidence indicates that viruses can become isolated and evolve along with their host species. This was first suggested for members of the Papovaviridae (Soeda et al., 1980) and was termed host-dependent evolution. N-terminal protein and gene sequences from occlusion body proteins of different baculoviruses suggested that a similar phenomenon was occurring with baculoviruses (Rohrmann et al., 1981; Rohrmann, 1992). Additionally, more comprehensive evidence from genome sequences has also provided support for this phenomenon (Herniou et al., 2004). These comparisons indicate that there are four main branches of baculoviruses. These include the GV and NPVs of Lepidoptera, the hymenopteran viruses, and the dipteran viruses. Such phylogenies should be viewed with caution, however, because they are currently based on a limited set of samples, e.g. sequence information for only one dipteran virus is available.

Genome structure

Over 20 unique baculovirus genomes have been sequenced and their diversity is reflected in their genome sizes which range from 82 kb in hymenopteran baculoviruses (Garcia-Maruniak et al., 2004; Lauzon et al., 2004) to almost 180 kb for a granulovirus (Hayakawa et al., 1999) and are predicted to encode from about 90 to 180 genes, respectively (Table 1). The high degree of size variability is caused by differences in gene content and numbers of repeated genes; some genes are repeated up to 17 times in a genome, and the extent of non-coding sequences (Hayakawa et al., 2000). A distinctive feature of many baculovirus genomes is the presence of homologous regions (*hrs*) located at a number of positions in the genome. In AcMNPV, the *hr* repeat units contain about 70-bp with an imperfect 30-bp palindrome near the center, and are repeated 2 to 8 times at each of eight locations around the genome (Fig. 1). Homologous regions are highly diverse and although they show

Table 1
Features of baculovirus genomes

Categories	Lepidoptera NPV/GV	Hymenoptera NPV	Diptera NPV
Genomes sequenced	16[a]/7	2	1
Genome size (kb)	101–178	82–86	108
ORFs (predicted)	119–181	90	109
Gene categories			
Replication			
DNA pol	+	+	+
LEF-1 (primase)	+	+	+
LEF-2 (primase assoc.)	+	+	+
Helicase	+	+	+
LEF-3 (SSB)	+	–	–
IE-1	+	–	–
DBP (SSB)	+	+	–
Alkaline nuclease	+	+	+
VLF-1	+	+	+
Transcription			
LEF-4 (capping enz.)	+	+	+
LEF-8	+	+	+
LEF-9	+	+	+
P47	+	+	+
BV envelope fusion			
GP64	+/–	–	–
F	+	–	+
Apoptosis			
P35	+/–	–	b
Iap	+	+	–

[a] Does not include closely related variants. Data as of 9/05.
[b] Although a dipteran NPV p35 homolog was reported (Afonso et al., 2001), it does not show up in a BLAST search using AcMNPV p35.

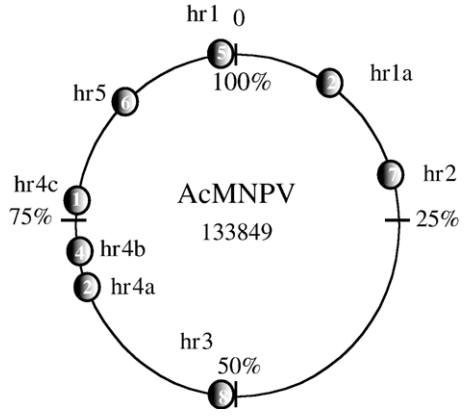

Fig. 1. Features of a baculovirus genome. Shown is a diagram of the AcMNPV genome. The homologous regions (*hrs*) are shown and represented as circles with numbers that indicate their location and the number of repeats present.

relatedness within a genome, they may show very limited homology between virus genomes both in their location and sequence. In a GV genome, for example, tandem repeated sequences are not found, although a 75-bp imperfect palindrome is present at 13 different locations on the genome (Luque et al., 1999). In addition, in one NPV sequence, *hrs* were not found (Willis et al., 2005). *Hrs* have been implicated both as transcriptional enhancers and origins of DNA replication for a number of baculoviruses (Guarino and Summers, 1986b; Guarino et al., 1986; Leisy and Rohrmann, 1993; Pearson and Rohrmann, 1995; Pearson et al., 1992, 1993).

The baculovirus infection cycle

Baculovirus occlusion bodies are ingested by insects and are dissolved by the high pH of their midguts. This releases the virions and they begin their infection by replicating in midgut cells. For most NPVs pathogenic for Diptera and Hymenoptera, the infection appears to be limited to gut cells (Federici, 1997; Moser et al., 2001; Young et al., 1972). In contrast, for most lepidopteran NPVs, the infection spreads from the midgut cells to a variety of other tissues via budded viruses (BV). BV differ from occluded virions (also called occlusion derived virus or ODV) in the structure of their envelope. Nucleocapsids destined to become BV are assembled in the nucleus and are observed within vesicles that bud off from the nuclear membrane into the cytoplasm. They appear to be released from the vesicles during their transit through the cytoplasm (Granados and Lawler, 1981) and subsequently they obtain their final envelope when they bud through a plasma membrane that has been modified by a virally encoded envelope fusion protein (see below). In contrast, ODV obtain their envelope within the nucleus and it may contain a number of virus encoded proteins (reviewed in (Funk et al., 1997)). The difference in BV and ODV envelope structure is reflected in their infectivity profile; BV are only poorly infectious by the oral infection route but are highly infectious for tissue culture cells. Likewise, ODV are of only very low infectivity for cultured cells, but are highly infectious via the oral route (Volkman and Summers, 1977).

Viral entry

Baculoviruses have two different envelope fusion proteins

After several different baculovirus genomes had been sequenced and all were found to encode homologs of the envelope fusion protein gp64, the lack of this gene in subsequent complete genome sequences such as that of a baculovirus (LdMNPV) pathogenic for the gypsy moth (*Lymantria dispar*) was unexpected (Kuzio et al., 1999). An open reading frame called LD130 was subsequently identified that had the features of a membrane protein including predicted signal and transmembrane domains. It was then demonstrated that constructs of LD130 were capable of mediating cell fusion at low pH when transfected into insect cells (Pearson et al., 2000). Similar observations were made for a related protein from another

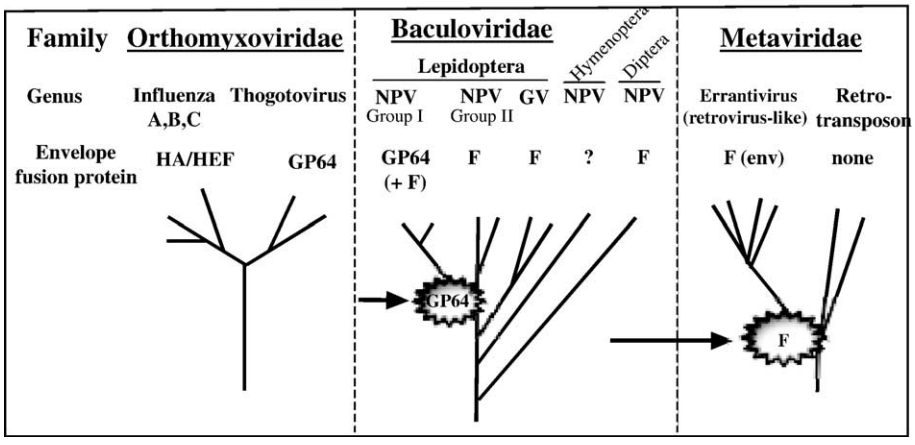

Fig. 2. Distribution of envelope fusion protein homologs. Three virus families are indicated. Below each viral family are listed genera or phylogenetic divisions present in the family and shown below that is the category of the envelope fusion protein. A schematic representation of the phylogenetic relationships (not to scale) of the genera is indicated at the bottom of the figure. The horizontal arrows show possible pathways of envelope fusion protein exchange. The gp64 arrow is not connected and reflects that gp64 may have entered a baculovirus lineage during a co-infection with a baculovirus and a thogotovirus or it could have entered both lineages independently via recombination with a gp64 homolog.

baculovirus (Ijkel et al., 2000). The LD130 family of proteins has been named 'F' or Fusion proteins. Subsequently, it was found that most baculoviruses lack homologs of GP64, but do have homologs of LD130 (reviewed in Pearson and Rohrmann, 2002). Therefore, the *gp64*-containing viruses encode homologs of two envelope fusion proteins, gp64 and F. Furthermore, it was observed that an F homolog in a *gp64*-containing virus localizes to both cell membranes and budded virus envelopes, but does not appear to mediate cell fusion (Pearson et al., 2002). In addition, it has been shown that when the F homolog in AcMNPV was deleted, virus replicated to normal titers in cultured cells, but larvae fed this virus showed a 28% reduction in mortality from the wt control (Lung et al., 2003). Because of the diversity of the F homologs and the contrasting high level of conservation of GP64 (>70% amino acid sequence identity) suggests that the F protein fusion function was displaced relatively recently in one branch of the baculoviruses by the incorporation of *gp64* (Fig. 2). Additional studies have demonstrated that F proteins can substitute for GP64 in AcMNPV (Lung et al., 2002). It is also of note that homologs of *gp64* are found not only in a branch of the Baculoviridae, but also in thogotoviruses, a genus of the Orthomyxoviridae (Morse et al., 1992; Portela et al., 1992) (Fig. 2).

Distribution of envelope fusion proteins within the Baculoviridae

The complete genome sequences for baculoviruses pathogenic for Lepidoptera and Diptera all contained homologs of the F group of envelope fusion proteins. Viruses that contain both GP64 and F homologs likely use GP64 as their envelope fusion protein and conversely lepidopteran viruses that lack GP64 likely use F homologs as their fusion protein. However, although this has been shown for two NPVs, it has not been demonstrated for the F proteins of GVs. In addition, substitution of a granulovirus homolog of F failed to rescue AcMNPV deleted for GP64 (Lung et al., 2002). The reason for this is not clear, but may indicate that GVs require an additional protein, or the cell lines tested are not compatible with the GV proteins. This observation could be related to the difficulty in developing cell culture systems for the growth of GVs (Winstanley and Crook, 1993).

Because of the widespread distribution of homologs of F in baculovirus genomes, the lack of genes encoding F proteins in sequences from two hymenopteran baculoviruses was unexpected (Garcia-Maruniak et al., 2004; Lauzon et al., 2004). Whereas the infections of the lepidopteran baculoviruses appear to cause systemic infections, those pathogenic for Diptera (Moser et al., 2001) and Hymenoptera (Young et al., 1972) replicate in the midgut and do not appear to cause systemic infection. However, the dipteran virus encodes an LD130 homolog (Afonso et al., 2001), whereas none were identified in the hymenopteran viruses. No other orfs have been implicated as budded virus fusion proteins in the hymenopteran virus genomes. It is not clear how the infection could be spread from cell to cell in the midgut if such a protein is lacking unless the envelope proteins used by occluded virions could be employed in this process. These proteins are not well characterized but if they are utilized, the virions might have to exit the cell nuclei with the envelope, be transferred into the lumen of the midgut, and then infect surrounding cells.

Envelope capture: a retrovirus env protein was likely obtained from a baculovirus

A second unexpected finding regarding baculovirus envelope fusion proteins was the observation that a group of insect retroviruses called the errantiviruses likely originated when a retrotransposon integrated into a baculovirus genome and obtained a copy of the baculovirus envelope protein gene via a recombination event. This resulted in the conversion of a retrotransposon into a retrovirus. Evidence for this event has been described independently in two reports (Malik et al., 2000; Rohrmann and Karplus, 2001) (reviewed in Pearson and Rohrmann, 2002). This observation was made particularly compelling by the earlier finding that an errantivirus could integrate into a baculovirus genome (Miller and Miller, 1982). Furthermore, subsequent research demonstrated that a furin-like cleavage site that was present in the baculovirus F proteins was conserved in an errantivirus env protein and was cleaved at this site (Pearson and Rohrmann, 2004). Therefore, evolutionary, mechanistic, and biochemical links support this relationship.

Other structural proteins

A number of structural proteins appear to be conserved among all baculoviruses. These include capsid-associated proteins such as vp39, p6.9, vp1054, and vp91 and a number of proteins associated with occluded virions including gp41; the tegument protein; and ODV envelope-associated proteins p74, odv-e56, and odv-ec27 (Garcia-Maruniak et al., 2004; Lauzon et al., 2004). Per os infectivity factors (PIF-1 and -2) also appear to be conserved. One of the most conserved structural proteins is polyhedrin/granulin and it shows over 46% amino acid identity between members from the lepidopteran and hymenopteran NPVs and GVs. However, surprisingly, the occlusion body protein from the one dipteran NPV sequence currently available shows no homology to the lepidopteran and hymenopteran NPV and GV polyhedrin/granulin sequences (Afonso et al., 2001).

Anti-apoptotic genes

The distribution and function of baculovirus anti apoptotic genes have been reviewed elsewhere (Clarke and Clem, 2003; Clem, 1997), therefore this section will be limited to a brief overview. The baculovirus anti-apoptotic inhibitor, p35, was originally identified in AcMNPV and subsequently was found to have an unexpected distribution. It is found in viruses closely related to AcMNPV (e.g. BmNPV) and a distinct variant has been found in *Spodoptera littoralis* nucleopolyhedrovirus (Du et al., 1999). The latter protein appears to block proteolytic activation of caspases at a unique step upstream from that

affected by P35 (Zoog et al., 2002). Most baculoviruses lack homologs of p35, but contain members of the inhibitor of apoptosis (iap) family which are metalloproteinases that block the apoptosis caspase cascade upstream of P35. They are found in up to four copies in baculovirus genomes. Although, in addition to p35, AcMNPV has two copies of iap genes, deletion of these genes did not appear to affect either AcMNPV host range or replication (Griffiths et al., 1999). In another virus that lacks a p35 homolog, four *iap* homologs were found, of which two could interfere with apoptosis (Maguire et al., 2000). Since the initial discovery of the baculovirus iap gene, homologs have been found to be widely distributed in eukaryotic organisms from yeast to humans.

Transcription

Baculovirus infections are initiated by the host RNA polymerase II which recognizes and transcribes early promoters which are similar to cellular promoters. Transcription is elevated by baculovirus transcriptional transactivators such as IE-1 (Guarino and Summers, 1986a) and *hr* sequences (see above). The early genes that are transcribed include a number that are involved in DNA replication. Concomitant with, or following DNA replication, late gene promoters are expressed by the baculovirus RNA polymerase. This polymerase recognizes the pentanucleotide promoter sequence A/G/T TAAG and initiation occurs at the first or second nucleotide within this sequence (Rankin et al., 1988; Rohrmann, 1986) (Fig. 3). The

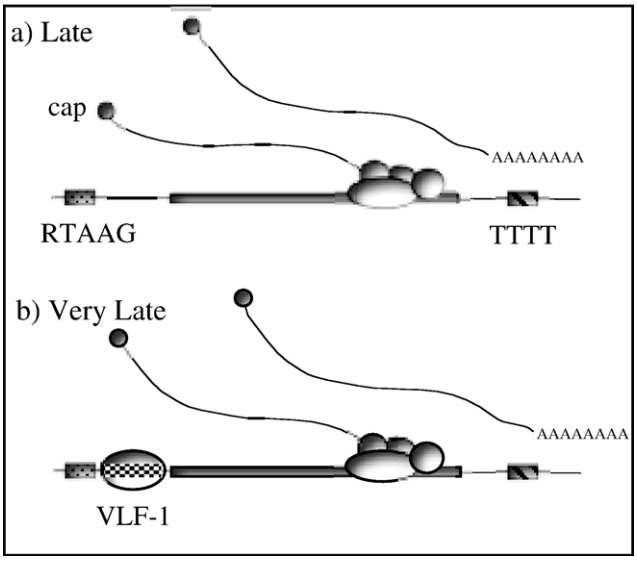

Fig. 3. Transcription of late and very late genes. (a) Late genes are initiated from the late promoter element RTAAG (*R* = A, G, or T) (stippled box). The polymerase complex contains four subunits including LEF-4 which likely caps the mRNA (represented by circles). A T-rich sequence (cross-hatched box) down stream of the late gene is thought to cause termination of RNA synthesis. (b) Very late genes have a similar promoter and termination signals but also contain an A–T-rich 'burst sequence' (checkerboard box) in-between the promoter and the beginning of the orf. This sequence binds VLF-1. This interaction is thought to facilitate the high level expression of very late genes. The burst sequence can also act as a translational enhancer in the very late p10 mRNA.

newly replicated DNA may be free of proteins that otherwise might inhibit the interaction of the promoter sequence with the late transcription complex. This is consistent with the ability to transcribe late promoter elements in vitro from deproteinated DNA templates (Glocker et al., 1993) and for the eventual shut off of late genes (e.g. vp39; Thiem and Miller, 1989) as the DNA becomes coated with proteins. The genes involved in late baculovirus gene transcription were originally identified using a late promoter-dependent transient assay system (Todd et al., 1995) and included a number that were also shown to be required for DNA replication using a transient replication assay (Kool et al., 1994; Lu and Miller, 1995). Subsequently, four of the gene products not associated with replication were found to co-purify as a complex and were able to support in vitro transcription from late promoter-containing DNA templates. This complex includes late expression factors (LEF) LEF-4, -8, -9, and p47 (Guarino et al., 1998). Homologs of these gene products have subsequently been identified in all baculovirus genomes that have been sequenced (Table 1). LEF-8 contains a conserved motif found in other RNA polymerases and it is thought that this is part of the catalytic site (Passarelli et al., 1994; Titterington et al., 2003). p47 was originally described by Carstens et al. (1993) and does not appear to be related to transcription-associated proteins from other organisms. LEF 9 contains a 7-amino acid motif (NTDCDGD or NRDCDGD except NADFDGD in the dipteran virus) similar to the Mg++ binding sequence (NADFDGD) found in the catalytic center in large RNA polymerase subunits of a number of DNA-dependent RNA polymerases (Lu and Miller, 1994). The D residues bind Mg++ and are conserved in all these sequences. LEF-4 was subsequently found to be an RNA capping enzyme (Gross and Shuman, 1998; Jin et al., 1998). The addition of an mRNA 5′ cap structure involves the hydrolysis of the gamma phosphate of 5′-triphosphate of the first nucleotide of pre-mRNA. The 5′ diphosphate end that results is capped by the transfer of GMP from GTP. This results in an inverted terminal dinucleotide structure G(5′)ppp(5′)N. The two reactions involve two different enzymatic activities; an RNA 5′ triphosphatase to remove the terminal gamma phosphate and the addition of GTP by guanylyltransferase. These two activities are present on a single protein located at the N- and C-termini, respectively, in metazoans and plants. Although having a similar activity, LEF-4 is unrelated to this category of capping enzyme, but is a member of a metal-dependent group of capping enzymes found in fungi and protozoa (Gross and Shuman, 1998).

The 5′ cap structure appears to serve two roles. It protects the 5′ end of the mRNA from degradation by exonucleases and it interacts with translation initiation factors thereby facilitating the initiation of translation. Capping in eukaryotes involves an enzyme that associates with the highly repetitive carboxy terminal domain (CTD) of the β′ subunit of RNA polymerase II. Because the baculovirus polymerase lacks a similar domain, it is likely that it evolved to include the enzyme as part of the RNA polymerase complex. However, assuming these reactions are free from exonuclease, it is not clear why LEF-4 is required for transcription in in vitro

assays. These assays monitor RNA transcripts that would not need to be capped to be detected. This suggests that LEF-4 may play a structural role in the organization of the polymerase subunits or it may have some other function. Complicating an interpretation of the role of LEF-4 in capping is evidence indicating that the 5′ untranslated region of p10 mRNA facilitates translation in a cap-independent manner (Scheper et al., 1997). Since commonly used internal ribosome entry sites are apparently not active in certain insect cell systems, the p10 5′ untranslated region has been used as a translational enhancer of uncapped mRNA in investigations employing insect cells (Kohl et al., 2004).

Termination and processing of late mRNAs

Early mRNAs are transcribed by the host RNA polymerase II and it is assumed that 3′ processing is carried out by enzymes associated with this complex. It was also assumed that baculoviruses used the host 3′ mRNA processing apparatus to cleave and polyadenylate late mRNAs downstream of the coding sequence. In one study, it was found that elimination of a polyadenylation signal downstream of the AcMNPV p10 did not influence expression levels, whereas replacing the region with a 3′ early terminator sequence from SV40 caused a reduction in expression levels (van Oers et al., 1999). Another investigation suggested that 3′ ends may be formed by the presence of T-rich sequences that destabilize the late transcription complex (Jin and Guarino, 2000) (Fig. 3). It is not clear how the ends are polyadenylated as this process is normally associated with 3′ cleavage by enzymes associated with the CTD present on the β′ subunit of eukaryotic RNA polymerases. As indicated above, this structure is not present in the baculovirus polymerase.

Very late gene expression

A novel feature of baculoviruses is their ability to express genes at high levels very late in infection. This phenomenon has been exploited in the development of baculoviruses as expression vectors. Two highly expressed very late genes have been characterized, polyhedrin and p10. Polyhedrin is the occlusion body protein, whereas the role of p10 is not clear, although it appears to form fibrillar structures that may be involved in the maturation of polyhedra (Russell et al., 1991) and the lysis of terminally infected nuclei (van Oers and Vlak, 1997). Because these genes appear to be involved in polyhedron morphogenesis, which is a very late step in the baculovirus life cycle that occurs after virions destined for occlusion have been assembled, it is likely that they are transcribed from DNA that does not become packaged as virion genomes thereby remaining accessible to the very late RNA polymerase complex. Both polyhedrin and p10 genes contain an A/T-rich sequence downstream of a late promoter sequence that is involved in their high level expression (Ooi et al., 1989). This sequence was called the ‘burst sequence’ because it caused a burst of transcription very late in infection (Fig. 4). Very late expression factor 1 (VLF-1) was originally identified because it influences the hyperexpression of very late genes (McLachlin and Miller, 1994). Subsequently, it was found that VLF-1 interacts with the burst sequence in gel shift assays (Yang and Miller, 1999). Homologs of VLF-1 are found in all sequenced baculovirus genomes and they belong to a family of proteins that includes lambda integrase. Integrases are a large group of site-specific DNA recombinases that catalyze DNA rearrangements and are found in a variety of organisms including viruses where they are involved in the integration and excision of viral genomes and decatenation of newly replicated chromosomes. Evidence suggests that VLF-1 may also be involved in the processing or packaging of baculovirus genomes (see below).

To date, VLF-1 is the only gene product identified that specifically influences very late gene expression. It is likely that high levels of gene expression are influenced by a number of features of baculovirus biology. These include: (i) the amplification of genes by DNA replication; (ii) the shut off of most late transcription, possibly by DNA binding proteins that coat the DNA and thereby free up RNA polymerase for very late transcription; (iii) the efficiency of the late polymerase and VLF-1 in recognizing and initiating from very late promoter elements; and (iv) the efficiency of LEF-4 in capping the mRNA. As mentioned above, the 5′ untranslated region of p10 mRNA appears to be capable of facilitating cap-independent translation (Scheper et al., 1997). Other factors that might enhance translation of very late expressed mRNAs have not been identified.

DNA replication

Identification of origins of viral DNA replication

In many small DNA viruses, replication initiates at a specific sequence and DNA replication results in the duplication of the genome. In contrast, baculoviruses, which have large genomes, present a more complex picture. Attempts to identify origins of replication using transient replication assays and the characterization of defective genomes have implicated homologous repeated sequences (*hrs*) (described above), non-*hr* sequences, and early promoters as origins of replication (Wu et al., 1999) (reviewed in Ahrens et al., 1996). Both a non-hr origin and an early promoter were shown to have origin activity in an in vivo pcr-based assay (Habib and Hasnain, 2000). These data suggest that replication might initiate at any site where the DNA becomes unwound and provides an entry point for the replication complex. The baculovirus transcriptional transactivator IE-1 binds to *hr* sequences and may be involved in facilitating entry at these sites. Likewise, RNA polymerase II would cause unwinding at early promoter sequences. Non-hr origins may provide sequences of less specificity that are involved in DNA unwinding.

If during normal virus replication DNA synthesis initiates at a variety of different locations, it would result in a heterogeneous population of daughter DNA molecules. This raises a number of major questions including how the completed genome is produced from DNA sequences with differing

beginning and end points and what is the rationale that led to this method of DNA replication.

Genome replication

Six virally encoded genes have been implicated as being essential for DNA replication in transient assays in AcMNPV (Kool et al., 1994; Lu and Miller, 1995). These include a primase (LEF-1) and a primase accessory factor (LEF-2), a helicase, a DNA polymerase, and a single-stranded DNA binding protein (SSB) called LEF-3 that can either anneal or unwind DNA depending on specific conditions (Mikhailov et al., 2005), and is also involved in the transport of helicase into the nucleus (Wu and Carstens, 1998; Chen and Carstens, 2005). The transcriptional transactivator IE-1 is also required for replication, although its role has not been determined.

Except for the SSB protein LEF-3 and the transactivator, IE-1, which are not found in hymenopteran or the dipteran virus, homologs of the primase complex, helicase, and DNA polymerase are found in all baculovirus genomes (Table 1). A second SSB, called DNA binding protein (DBP) (Mikhailov et al., 1998), is found in all baculovirus genomes with the exception of the dipteran virus. In addition to LEF-3, LdMNPV encodes two copies of DBP that have about 26% amino acid identity (Kuzio et al., 1999). The roles of LEF-3 in AcMNPV DNA replication have been studied, but the role of DBP, which appears to be expressed at much higher levels than LEF-3 (Mikhailov et al., 1998), is not clear.

Although the gene products identified for AcMNPV can synthesize DNA, a variety of other proteins are required for the production of complete infectious genomes. Research that we have initiated on an AcMNPV orf encoding an alkaline nuclease has shed light on its possible role in the production of complete viral genomes. Homologs of alkaline nuclease are found in all sequenced baculovirus genomes reflecting its likely importance in their biology. In addition, it is related to a nuclease family with members found in a variety of viruses from lambda phage to the Herpesviridae. In these viruses, it associates with a single-stranded DNA binding protein. Similarly, the alkaline nuclease (AN) of AcMNPV is associated with the SSB protein, LEF-3 (Mikhailov et al., 2003). These nucleases digest linear DNA from the ends in a 5′ to 3′ direction and result in a single-stranded tail comprised of the 3′ strand of the DNA. These single-stranded sequences can form hybrids with homologous sequences thereby generating longer sequences. In addition, by strand invasion at regions of homology on another double-stranded DNA sequence, the single-stranded ends can generate replication forks at sites throughout the genome. Our results indicate that when the *an* gene is deleted from the viral genome, although DNA is replicated (Okano et al., 2004), much of the DNA is smaller than genome length (unpublished) suggesting that recombination may play a major role in the production of higher MW DNA (Fig. 4). Recombination has also been implicated in replication of some other DNA viruses such as T4, lambda, and herpes viruses.

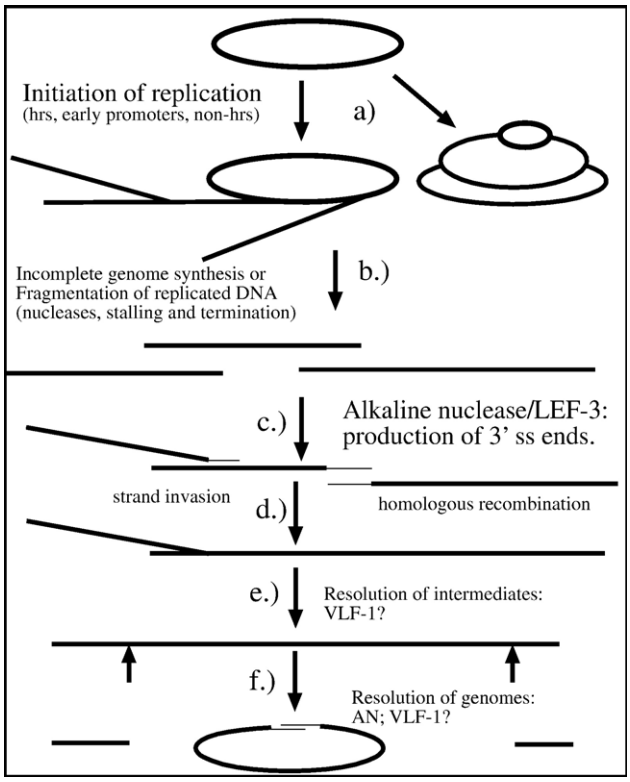

Fig. 4. A model for baculovirus genome replication. (a) Baculovirus DNA replication appears to be initiated at a variety of different types of sequences including *hr*, non-*hr*, and RNA polymerase II promoters. Replication may occur via two pathways shown; rolling circle or a theta mode. (b) Many of the sequences generated may be smaller than complete genomes. This might be caused by either nuclease action or stalling and disassembly of the complexes. (c) The genome fragments are acted upon by the 5′ to 3′ nuclease activity of alkaline nuclease resulting in 3′ single-stranded tails. These tails are involved in DNA recombination either by the annealing of homologous single strands, or by strand invasion. (d) Homologous recombination and subsequent processing results in larger than genome size molecules. (e) These molecules are then processed into mature genomes. Evidence suggests that VLF-1 is involved in this latter process.

Implications of recombination-dependent replication

There are a number of implications of recombination-dependent replication that are related to properties of baculoviruses.

The use of multiple replication origins

The use of multiple replication origins may greatly amplify the amount of viral DNA that can be produced in a given time. The constraints of identifying and initiating replication at a specific site would be avoided and replication could originate simultaneously at a number of sites. Upon recombination, molecules destined to become genomes would be produced that could begin or end anywhere as long as they were greater than genome length.

Resolution of subgenome size or broken DNA

It is possible that baculoviruses may produce subgenome size DNA as part of their normal replication and recombination acts on these fragments to produce complete genomes. However, the production of less than genome size DNA may

be a result of the cellular milieu that may contain a high level of nucleases that can nick or completely cleave nascent viral DNA. Furthermore, the size of baculovirus genomes may pose inherent challenges to complete replication because of the probability that the replication fork may be damaged or nicked during replication or the complex may stall and detach from the DNA before genome replication can be completed. Under both these circumstances, incomplete genomes would result. Therefore, the use of multiple replication origins and the problem of nuclease damage and/or replication processivity, may have led to the evolution of a recombination-based method of replication in order to ensure the rapid amplification of large segments of DNA.

The covalently closed circular genome

The production of covalently closed circular DNA could be mediated by a final recombination event in which two homologous areas near the ends of a greater than unit length linear replication intermediate are joined.

The production of defective interfering particles

Recombination-based replication also has a number of other implications. The production of defective interfering particles likely results from the recombination of incomplete fragments during recombinational assembly of genomes rather than by deletions per se.

The facility with which baculovirus genomes recombine

Recombination was the basis for the ability to generate baculoviruses for use as expression vectors.

Baculovirus diversity

Baculoviruses show a high degree of variation in gene content (Herniou et al., 2003) and this likely reflects the incorporation of genes from their host insect and other viruses. Recombination-dependent replication could greatly increase the frequency of these events.

The production of multiple nucleocapsids

Certain lepidopteran NPVs produce occluded virions in which multiple nucleocapsids are present within a single envelope. This may lead the initiation of infection by several nucleocapsids and may facilitate recombination and repair of those that might be damaged (Blissard and Rohrmann, 1989). Such damage could occur during their production, after they are released, or during infection in the midgut or within cells. The elucidation of the role of recombination in baculovirus DNA replication provides further support for this theory.

The lack of genome isomerization

Similar to baculoviruses, herpes simplex virus I (HSV-1) and other herpes viruses are highly recombinogenic (reviewed in Thiry et al., 2005; Wilkinson and Weller, 2003). During HSV-1 replication, intra-genomic inversions between three repeated elements within the genome result in a population of four different genome isomers. Since most baculovirus genomes appear to be punctuated with homologous repeated sequences that are distributed throughout their genomes (e.g. AcMNPV has 7 *hrs*, see above and Fig. 1), it is surprising that their genomes are replicated with such fidelity. It is clear that such inversions do occur as there are examples of inversions bracketed by *hrs* between species (e.g. AcMNPV orfs 1–10 are bordered by *hr*1 and *hr*1a and are inverted relative to the homologous sequences in the *Orgyia pseudotsugata* MNPV genome (Ahrens et al., 1997). However, there is no evidence that major populations of isomers are packaged into virions during normal virus replication. Restriction enzyme digestion of baculovirus genomic DNA results in a single characteristic pattern for each enzyme and that pattern conforms to the sequence of the genome. Since one might expect that *hr* inversions and other forms of recombination between these elements would be common during baculovirus replication, a mechanism must exist to either minimize these events or to eliminate such recombinants from the genome population.

Processing of genome-size DNA

Although recombination-based replication appears to solve a number of problems that are confronted by the replication of baculovirus genomes, it might result in complex branched structures that would have to be resolved into covalently closed circular genomes of unit length. The mechanism leading to the resolution of these structures is not clear. One protein, very late factor-1 (VLF-1), has been implicated in this process. VLF-1 homologs are found in all baculovirus genomes sequenced to date (~25 total) and it was originally identified because it influences the hyperexpression of very late genes (McLachlin and Miller, 1994). It is a member of the lambda integrase (Int) family of proteins. Members of this integrase family recombine DNA duplexes via two consecutive strand break and ligation steps along with topoisomerization of the substrates (Esposito and Scocca, 1997). A feature of these enzymes is that a conserved tyrosine forms a covalent link with DNA during the cleavage process. Alteration of this tyrosine in AcMNPV VLF-1 appeared to be lethal to the virus (Yang and Miller, 1998). Further evidence that VLF-1 may be involved in DNA processing is its location at one end of capsids (Vanarsdall et al., unpublished) and its ability to bind with high affinity to cruciform DNA that mimics a structure common to recombination intermediates (Mikhailov and Rohrmann, 2002). In addition, when VLF-1 is deleted from the virus genome, DNA synthesis occurs, but no viable virus is produced (Vanarsdall et al., 2004). The endonucleolytic activity of the baculovirus alkaline nuclease (Mikhailov et al., 2004) could also be involved in processing branched DNA intermediates.

DNA replication and nucleocapsid assembly

Early in the infection, DNA replication may result in the rapid amplification of DNA that is used as a template for the expression of late genes that encode a variety of proteins including those involved in capsid structure. However, once capsids begin to be assembled and the concentration of viral

DNA increases, genome production may be coordinated with its packaging into preformed capsids. The pre-assembly of capsids has been suggested by electron microscopy (Fraser, 1986). In addition, most of the proteins that have been implicated in DNA replication and processing including DNA polymerase, helicase, IE-1, LEF-3, LEF-1, VLF-1, and alkaline nuclease have been reported to be associated with purified occluded baculovirus nucleocapsids (Braunagel et al., 2003). This association may reflect macromolecular complexes that are 'frozen' in association with nucleocapsids by the occlusion process (in contrast, these complexes could be lost from BV during its transit through the nuclear membrane and cytoplasm before budding from the cytoplasmic membrane). The presence of these replication-associated proteins with the nucleocapsid could reflect a highly coordinated set of reactions including DNA synthesis, recombination, processing, and packaging in close proximity to the nucleocapsids. The insertion of DNA into nucleocapsids as it is synthesized could protect the partially packaged DNA from strand invasion or nuclease attack. Such events would be confined to ends of the DNA that are outside the capsid. It is not known what drives the insertion of the genome into the capsids. If the capsids are pre-assembled, it is likely that an ATP-dependent molecular motor is involved in propelling and compacting the DNA into the capsids as has been demonstrated for other viruses (Smith et al., 2001). It has been noted that during infection, actin which is normally in the cytoplasm, is transported to the nucleus (Volkman et al., 1992) and interacts with structural components of the virion (Lanier and Volkman, 1998). In addition, reagents that block actin polymerization may result in the production of aberrant capsids that lack DNA (Volkman, 1988). Collectively, these observations indicate that components of the cytoskeleton could be involved in the insertion of DNA into nucleocapsids.

Nucleocapsid length/genome size

Another major unanswered question involves the parameters that determine the length of the capsid and the size of the DNA molecule that is packaged. An examination of capsids associated with defective viral genomes suggested that capsid length may be flexible in response to genome size (Kool et al., 1991). If capsids are both pre-assembled and can vary in length, it would suggest that they can be expanded or reduced in response to the size of the genome as part of the packaging process. The facility with which baculoviruses can be engineered to contain additional genetic material could also indicate that a unit size capsid may have some flexibility in the length of DNA that can be accommodated. How the virus senses that a genome is complete and terminates the encapsidation process remains to be determined.

Conclusions: what defines members of the Baculoviridae?

Members of the baculoviridae appear to share a number of features. They infect insects and are occluded, they have rod shaped virions, and a double-stranded circular DNA genome. Based on the conservation of open reading frames, it is likely that a number of the molecular processes involved in late gene expression and DNA replication are similar. In particular, they all appear to share a core set of genes involved in late gene transcription. In addition, they also share a core set of genes (DNA polymerase, the primase complex, and helicase) involved in DNA replication, although there may be some variation in the SSB and transactivator components. Homologs of two proteins implicated in genome processing, alkaline nuclease and very late expression factor-1 (VLF-1), are present in all baculovirus genomes suggesting common mechanisms for this process. The envelope fusion proteins that are involved in viral entry provide a fascinating example of evolution of these important molecules. Baculoviruses appear to have been involved as both a recipient (gp64) and a donor (F protein to the errantiviruses) of these proteins. Similar to envelope fusion proteins is the presence of anti-apoptotic inhibitors. All baculoviruses appear to encode homologs of one or both categories of these proteins.

A group of persistent viruses shows some similarities to the Baculoviridae. They infect insects, have rod-shaped virions, and DS DNA, but they are not occluded and at 228 kb the genome of the single example sequenced is significantly larger than known baculoviruses, and they are currently unclassified (Cheng et al., 2002). The single genome sequenced was found to encode homologs of the *vlf-1*, *lef-8*, *iap*, and several structural proteins. However, the current understanding of this virus is too limited to include it within the Baculoviridae.

Acknowledgment

This research was supported by a grant from the NIH (GM060404) to G.F.R.

References

Afonso, C.L., Tulman, E.R., Lu, Z., Balinsky, C.A., Moser, B.A., Becnel, J.J., Rock, D.L., Kutish, G.F., 2001. Genome sequence of a baculovirus pathogenic for *Culex nigripalpus*. J. Virol. 75, 11157–11165.

Ahrens, C.A., Leisy, D.J., Rohrmann, G.F., 1996. Baculovirus DNA replication. In: De Pamphilis, M. (Ed.), DNA Replication in Eukaryotic Cells. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 855–872.

Ahrens, C.H., Russell, R., Funk, C.J., Evans, J.T., Harwood, S.H., Rohrmann, G.F., 1997. The sequence of the *Orgyia pseudotsugata* multinucleocapsid nuclear polyhedrosis virus genome. Virology 229, 381–399.

Blissard, G.W., Rohrmann, G.F., 1989. Location, sequence, transcriptional mapping, and temporal expression of the gp64 envelope glycoprotein gene of the *Orgyia pseudotsugata* multicapsid nuclear polyhedrosis virus. Virology 170, 537–555.

Braunagel, S.C., Russell, W.K., Rosas-Acosta, G., Russell, D.H., Summers, M.D., 2003. Determination of the protein composition of the occlusion-derived virus of *Autographa californica* nucleopolyhedrovirus. Proc. Natl. Acad. Sci. U.S.A. 100 (17), 9797–9802.

Carstens, E.B., Lu, A.L., Chan, H.L.B., 1993. Sequence, transcriptional mapping, and overexpression of p47, a baculovirus gene regulating late gene expression. J. Virol. 67, 2513–2520.

Chen, Z., Carstens, E.B., 2005. Identification of domains in Autographa californica multiple nucleopolyhedrovirus late expression factor 3 required for nuclear transport of P143. J. Virol. 79, 10915–10922.

Cheng, C.H., Liu, S.M., Chow, T.Y., Hsiao, Y.Y., Wang, D.P., Huang, J.J.,

Chen, H.H., 2002. Analysis of the complete genome sequence of the Hz-1 virus suggests that it is related to members of the baculoviridae. J. Virol. 76, 9024–9034.

Clarke, T.E., Clem, R.J., 2003. Insect defenses against virus infection: the role of apoptosis. Int. Rev. Immunol. 22 (5–6), 401–424.

Clem, R.J., Regulation of Programmed Cell Death by Baculoviruses. The Baculoviruses. Plenum. (Edited by L.K. Miller).

Du, Q., Lehavi, D., Faktor, O., Qi, Y., Chejanovsky, N., 1999. Isolation of an apoptosis suppressor gene of the *Spodoptera littoralis* nucleopolyhedrovirus. J. Virol. 73, 1278–1285.

Esposito, D., Scocca, J.J., 1997. The integrase family of tyrosine recombinases: evolution of a conserved active site domain. Nucleic Acids Res. 25 (18), 3605–3614.

Federici, B.A., 1997. Baculovirus pathogenesis. In: Miller, L.K. (Ed.), The Baculoviruses. Plenum, New York.

Fraser, M.J., 1986. Ultrastructural observations of virion maturation in *Autographa californica* nuclear polyhedrosis virus infected *Spodoptera frugiperda* cell cultures. J. Ultrastruct. Mol. Struct. Res. 95, 189–195.

Funk, C.J., Braunagel, S., Rohrmann, G.F., 1997. Baculovirus structure. In: Miller, L.K. (Ed.), The Baculoviruses. Plenum, pp. 2–32.

Garcia-Maruniak, A., Maruniak, J.E., Zanotto, P.M.A., Doumbouya, A.E., Liu, J.-C., Merritt, T.M., Lanoie, J.S., 2004. Sequence analysis of the genome of the *Neodiprion sertifer* nucleopolyhedrovirus. J. Virol. 78, 7036–7051.

Glocker, B., Hoopes Jr., R.R., Hodges, L., Rohrmann, G.F., 1993. In vitro transcription from baculovirus late gene promoters: accurate mRNA initiation by nuclear extracts prepared from infected *Spodoptera frugiperda* cells. J. Virol. 67, 3771–3776.

Granados, R.R., Lawler, K.A., 1981. In vivo pathway of *Autographa californica* baculovirus invasion and infection. Virology l08, 297–308.

Griffiths, C.M., Barnett, A.L., Ayres, M.D., Windass, J., King, L.A., Possee, R.D., 1999. In vitro host range of *Autographa californica* nucleopolyhedrovirus recombinants lacking functional p35, iap1 or iap2. J. Gen. Virol. 80, 1055–1066.

Gross, C.H., Shuman, S., 1998. RNA 5′-triphosphatase, nucleoside triphosphatase, and guanylyltransferase activities of baculovirus LEF-4 protein. J. Virol. 72 (12), 10020–10028.

Guarino, L.A., Summers, M.D., 1986a. Functional mapping of a trans-activating gene required for expression of a baculovirus delayed-early gene. J. Virol. 57, 563–571.

Guarino, L.A., Summers, M.D., 1986b. Interspersed homologous DNA of *Autographa californica* nuclear polyhedrosis virus enhances delayed-early gene expression. J. Virol. 60, 215–223.

Guarino, L.A., Gonzalez, M.A., Summers, M.D., 1986. Complete sequence and enhancer function of the homologous DNA regions of *Autographa californica* nuclear polyhedrosis virus. J. Virol. 60, 224–229.

Guarino, L.A., Xu, B., Jin, J., Dong, W., 1998. A virus-encoded RNA polymerase purified from baculovirus-infected cells. J. Virol. 72, 7985–7991.

Habib, S., Hasnain, S.E., 2000. Differential activity of two non-hr origins during replication of the baculovirus *Autographa californica nuclear* polyhedrosis virus genome. J. Virol. 74 (11), 5182–5189.

Hayakawa, T., Ko, R., Okano, K., Seong, S., Goto, C., Maeda, S., 1999. Sequence analysis of the *Xestia c-nigrum* granulovirus genome. Virology 262, 277–297.

Hayakawa, T., Rohrmann, G.F., Hashimoto, Y., 2000. Patterns of genome organization and content in lepidopteran baculoviruses. Virology 278, 1–12.

Herniou, E.A., Olszewski, J.A., Cory, J.S., O'Reilly, D.R., 2003. The genome sequence and evolution of baculoviruses. Annu. Rev. Entomol. 48, 211–234.

Herniou, E.A., Olszewski, J.A., O'Reilly, D.R., Cory, J.S., 2004. Ancient coevolution of baculoviruses and their insect hosts. J. Virol. 78 (7), 3244–3251.

Ijkel, W.F.J., Westernberg, M., Goldbach, R.W., Blissard, G.W., Vlak, J.M., Zuidema, D., 2000. A novel baclovirus envelope fusion protein with a proprotein convertase cleavage site. Virology 274, 30–41.

Jin, J., Guarino, L.A., 2000. 3′-end formation of baculovirus late RNAs. J. Virol. 74 (19), 8930–8937.

Jin, J., Dong, W., Guarino, L.A., 1998. The LEF-4 subunit of baculovirus RNA polymerase has RNA 5′-triphosphatase and ATPase activities. J. Virol. 72 (12), 10011–10019.

Kohl, A., Hart, T.J., Noonan, C., Royall, E., Roberts, L.O., Elliott, R.M., 2004. A bunyamwera virus minireplicon system in mosquito cells. J. Virol. 78 (11), 5679–5685.

Kool, M., Voncken, J.W., Van Lier, F.L.J., Tramper, J., Vlak, J.M., 1991. Detection and analysis of *Autographa californica* nuclear polyhedrosis virus mutants with defective interfering properties. Virology 183, 739–746.

Kool, M., Ahrens, C., Goldbach, R.W., Rohrmann, G.F., Vlak, J.M., 1994. Identification of genes involved in DNA replication of the *Autographa californica* baculovirus. Proc. Natl. Acad. Sci. U.S.A. 91, 11212–11216.

Kuzio, J., Pearson, M.N., Harwood, S.H., Funk, C.J., Evans, J.T., Slavicek, J., Rohrmann, G.F., 1999. Sequence and analysis of the genome of a baculovirus pathogenic for *Lymantria dispar*. Virology 253, 17–34.

Lanier, L.M., Volkman, L.E., 1998. Actin binding and nucleation by *Autographa californica* M nucleopolyhedrovirus. Virology 243 (1), 167–177.

Lauzon, H.A.M., Lucarotti, C.J., Krell, P.J., Feng, Q., Retnakaran, A., Arif, B.M., 2004. Sequence and organization of the *Neodiprion lecontei* nucleopolyhedrovirus genome. J. Virol. 78, 7023–7035.

Leisy, D.J., Rohrmann, G.F., 1993. Characterization of the replication of plasmids containing *hr* sequences in baculovirus-infected *Spodoptera frugiperda* cells. Virology 196, 722–730.

Lu, A., Miller, L.K., 1994. Identification of three late expression factor genes within the 33.8- to 43.4-map-unit region of *Autographa californica* nuclear polyhedrosis virus. J. Virol. 68, 6710–6718.

Lu, A., Miller, L.K., 1995. The roles of eighteen baculovirus late expression factor genes in transcription and DNA replication. J. Virol. 69, 975–982.

Lung, O., Westenberg, M., Vlak, J.M., Zuidema, D., Blissard, G.W., 2002. Pseudotyping *Autographa californica* multicapsid nucleopolyhedrovirus (AcMNPV): F proteins from group II NPVs are functionally analogous to AcMNPV GP64. J. Virol. 76, 5729–5736.

Lung, O.Y., Cruz-Alvarez, M., Blissard, G.W., 2003. Ac23, an envelope fusion protein homolog in the baculovirus *Autographa californica* multicapsid nucleopolyhedrovirus, is a viral pathogenicity factor. J. Virol. 77 (1), 328–339.

Luque, T., Finch, R., Crook, N., O'Reilly, D., Winstanley, D., 2001. The complete sequence of the Cydia pomonella granulovirus genome. J. Gen. Virol. 82, 2531–2547.

Maguire, T., Harrison, P., Hyink, O., Kalmakoff, J., Ward, V.K., 2000. The inhibitors of apoptosis of *Epiphyas postvittana* nucleopolyhedrovirus. J. Gen. Virol. 81 (11), 2803–2811.

Malik, H.S., Henikoff, S., Eickbush, T.H., 2000. Poised for contagion: evolutionary origins of the infectious abilities of invertebrate retroviruses. Genome Res. 10, 1307–1318.

McLachlin, J.R., Miller, L.K., 1994. Identification and characterization of vlf-1, a baculovirus gene involved in very late gene expression. J. Virol. 68, 7746–7756.

Mikhailov, V.S., Rohrmann, G., 2002. Binding of the baculovirus very late expression factor 1 (VLF-1) to different DNA structures. BMC Mol. Biol. 3, 14.

Mikhailov, V.S., Mikhailova, A.L., Iwanaga, M., Gomi, S., Maeda, S., 1998. *Bombyx mori* nucleopolyhedrovirus encodes a DNA-binding protein capable of destabilizing duplex DNA. J. Virol. 72, 3107–3116.

Mikhailov, V., Okano, K., Rohrmann, G., 2003. Baculovirus alkaline nuclease possesses a 5′→3′ exonuclease activity and associates with the DNA-binding protein LEF-3. J. Virol. 77 (4), 2436–2444.

Mikhailov, V., Okano, K., Rohrmann, G., 2004. Specificity of the endonuclease activity of the baculovirus alkaline nuclease for single-stranded DNA. J. Biol. Chem. 279 (15), 14734–14745.

Mikhailov, V.S., Okano, K., Rohrmann, G.F., 2005. The redox state of the baculovirus single-stranded DNA-binding protein LEF-3 regulates its DNA binding, unwinding, and annealing activities. J. Biol. Chem. 280, 29444–29453.

Miller, D.W., Miller, L.K., 1982. A virus mutant with an insertion of a copia-like transposable element. Nature (London) 299, 562–564.

Morse, M.A., Marriott, A.C., Nuttall, P.A., 1992. The glycoprotein of Thogoto

virus (a tick-borne orthomyxo-like virus) is related to the baculovirus glycoprotein gp64. Virology 186, 640–646.
Moser, B., Becnel, J., White, S., Afonso, C., Kutish, G., Shanker, S., Almira, E., 2001. Morphological and molecular evidence that *Culex nigripalpus* baculovirus is an unusual member of the family Baculoviridae. J. Gen. Virol. 82, 283–297.
Okano, K., Vanarsdall, A.L., Rohrmann, G.F., 2004. Characterization of a baculovirus lacking the alkaline nuclease gene. J. Virol. 78, 10650–10656.
Ooi, B.G., Rankin, C., Miller, L.K., 1989. Downstream sequences augment transcription from the essential initiation site of a baculovirus polyhedrin gene. J. Mol. Biol. 210, 721–736.
O'Reilly, D.R., Winstanley, D., 2001. The complete sequence of the Cydia pomonella granulovirus genome. J. Gen. Virol. 82, 2531–2547.
Passarelli, A.L., Todd, J.W., Miller, L.K., 1994. A baculovirus gene involved in late gene expression predicts a large polypeptide with a conserved motif of RNA polymerases. J. Virol. 68, 4673–4678.
Pearson, M.N., Rohrmann, G.F., 1995. *Lymantria dispar* nuclear polyhedrosis virus homologous regions: characterization of their ability to function as replication origins. J. Virol. 69, 213–221.
Pearson, M.N., Rohrmann, G.F., 2002. Transfer, incorporation, and substitution of envelope fusion proteins among members of the Baculoviridae, Orthomyxoviridae, and Metaviridae (insect retrovirus) families. J. Virol. 76, 5301–5304.
Pearson, M.N., Rohrmann, G.F., 2004. Conservation of a proteinase cleavage site between an insect retrovirus (gypsy) env protein and a baculovirus envelope fusion protein. Virology 322 (1), 61–68.
Pearson, M.N., Bjornson, R.M., Pearson, G.D., Rohrmann, G.F., 1992. The *Autographa californica* baculovirus genome: evidence for multiple replication origins. Science 257, 1382–1384.
Pearson, M.N., Bjornson, R.M., Ahrens, C., Rohrmann, G.F., 1993. Identification and characterization of a putative origin of DNA replication in the genome of a baculovirus pathogenic for *Orgyia pseudotsugata*. Virology 197, 715–725.
Pearson, M.N., Groten, C., Rohrmann, G.F., 2000. Identification of the *Lymantria dispar* nucleopolyhedrovirus envelope fusion protein provides evidence for a phylogenetic division of the Baculoviridae. J. Virol. 74, 6126–6131.
Pearson, M.N., Russell, R.L.Q., Rohrmann, G.F., 2002. Functional analysis of a conserved region of the baculovirus envelope fusion protein, LD130. Virology 304, 81–88.
Portela, A., Jones, L.D., Nuttall, P., 1992. Identification of viral structural polypeptides of Thogoto virus (a tick-borne orthomyxo-like virus) and functions associated with the glycoprotein. J. Gen. Virol. 73 (Pt. 11), 2823–2830.
Rankin, C., Ooi, B.G., Miller, L.K., 1988. Eight base pairs encompassing the transcriptional start point are the major determinant for baculovirus polyhedrin gene expression. Gene 70, 39–49.
Rohrmann, G.F., 1986. Polyhedrin structure. J. Gen. Virol. 67, 1499–1513.
Rohrmann, G.F., 1992. Baculovirus structural proteins. J. Gen. Virol. 73, 749–761.
Rohrmann, G.F., 1999. Nuclear polyhedrosis viruses. In: Webster, R.G., Granoff, A. (Eds.), Encyclopedia of Virology, 2nd ed. Academic Press, London, pp. 146–152.
Rohrmann, G.F., Karplus, P.A., 2001. Relatedness of baculovirus and gypsy retrotransposon envelope proteins. BMC Evol. Biol. 1, 1.
Rohrmann, G., Pearson, M., Bailey, T., Becker, R., Beaudreau, G., 1981. N-terminal polyhedrin sequences and occluded Baculovirus evolution. J. Mol. Evol. 17, 329–333.
Russell, R.L.Q., Pearson, M.N., Rohrmann, G.F., 1991. Immunoelectron microscopic examination of *Orgyia pseudotsugata* multicapsid nuclear polyhedrosis virus-infected *Lymantria dispar* cells: time course and localization of major polyhedron-associated proteins. J. Gen. Virol. 72, 275–283.
Scheper, G.C., Vries, R.G., Broere, M., Usmany, M., Voorma, H.O., Vlak, J.M., Thomas, A.A., 1997. Translational properties of the untranslated regions of the p10 messenger RNA of *Autographa californica* multicapsid nucleopolyhedrovirus. J. Gen. Virol. 78 (3), 687–696.
Smith, D.E., Tans, S.J., Smith, S.B., Grimes, S., Anderson, D.L., Bustamante, C., 2001. The bacteriophage phi29 portal motor can package DNA against a large internal force. Nature 413 (6857), 748–752.
Soeda, E., Maruyama, T., Arrand, J., Griffin, B., 1980. Host-dependent evolution of three papova viruses. Nature 285, 165–167.
Thiem, S.M., Miller, L.K., 1989. Identification, sequence, and transcriptional mapping of the major capsid protein gene of the baculovirus *Autographa californica* nuclear polyhedrosis virus. J. Virol. 63, 2008–2018.
Thiry, E., Meurens, F., Muylkens, B., McVoy, M., Gogev, S., Thiry, J., Vanderplasschen, A., Epstein, A., Keil, G., Schynts, F., 2005. Recombination in alphaherpesviruses. Rev. Med. Virol. 15 (2), 89–103.
Titterington, J.S., Nun, T.K., Passarelli, A.L., 2003. Functional dissection of the baculovirus late expression factor-8 gene: sequence requirements for late gene promoter activation. J. Gen. Virol. 84 (7), 1817–1826.
Todd, J.W., Passarelli, A.L., Miller, L.K., 1995. Eighteen baculovirus genes, including *lef-11*, *p35*, *39K*, and *p47*, support late gene expression. J. Virol. 69, 968–974.
Vanarsdall, A.L., Okano, K., Rohrmann, G.F., 2004. Characterization of a baculovirus with a deletion of *vlf-1*. Virology 326 (1), 191–201.
van Oers, M.M., Vlak, J.M., 1997. The baculovirus 10-kDa protein. J. Invertebr. Pathol. 70 (1), 1–17.
van Oers, M.M., Vlak, J.M., Voorma, H.O., Thomas, A.A., 1999. Role of the 3′ untranslated region of baculovirus p10 mRNA in high-level expression of foreign genes. J. Gen. Virol. 80, 2253–2262.
Volkman, L.E., 1988. *Autographa californica* MNPV nucleocapsid assembly: inhibition by cytochalasin D. Virology 163 (2), 547–553.
Volkman, L.E., Summers, M.D., 1977. *Autographa californica* nuclear polyhedrosis virus: comparative infectivity of the occluded, alkali-liberated, and nonoccluded forms. J. Invertebr. Pathol. 30, 102–103.
Volkman, L.E., Talhouk, S.N., Oppenheimer, D.I., Charlton, C.A., 1992. Nuclear F-actin: a functional component of baculovirus-infected lepidopteran cells? J. Cell Sci. 103, 15–22.
Wilkinson, D.E., Weller, S.K., 2003. The role of DNA recombination in herpes simplex virus DNA replication. IUBMB Life 55 (8), 451–458.
Willis, L.G., Siepp, R., Stewart, T.M., Erlandson, M.A., Theilmann, D.A., 2005. Sequence analysis of the complete genome of *Trichoplusia ni* single nucleopolyhedrovirus and the identification of a baculoviral photolyase gene. Virology 338, 209–226.
Winstanley, D., Crook, N.E., 1993. Replication of *Cydia pomonella* granulosis virus in cell cultures. J. Gen. Virol. 74 (8), 1599–1609.
Winstanley, D., O'Reilly, D., 1999. Granuloviruses. In: Webster, R.G., Granoff, A. (Eds.), Encyclopedia of Virology, 2nd ed. Academic Press, London, pp. 127–130.
Wu, Y., Carstens, E.B., 1998. A baculovirus single-stranded DNA binding protein, LEF-3, mediates the nuclear localization of the putative helicase P143. Virology 247, 32–40.
Wu, Y., Liu, G., Carstens, E.B., 1999. Replication, integration, and packaging of plasmid DNA following cotransfection with baculovirus viral DNA. J. Virol. 73 (7), 5473–5480.
Yang, S., Miller, L.K., 1998. Expression and mutational analysis of the baculovirus very late factor 1 (vlf-1) gene. Virology 245 (1), 99–109.
Yang, S., Miller, L.K., 1999. Activation of baculovirus very late promoters by interaction with very late factor 1. J. Virol. 73 (4), 3404–3409.
Young, S.Y., Livingston, J.M., McMasters, J.A., Yearian, W.C., 1972. A nuclear polyhedrosis virus of the loblolly pine sawfly, *Neodiprion taedae* linearis ross. J. Invertebr. Pathol. 20 (2), 220–221.
Zoog, S.J., Schiller, J.J., Wetter, J.A., Chejanovsky, N., Friesen, P.D., 2002. Baculovirus apoptotic suppressor P49 is a substrate inhibitor of initiator caspases resistant to P35 in vivo. EMBO J. 21 (19), 5130–5140.